24. (New) The method of claim 12, wherein said discrete, detectable spots are separately quantifiable.

REMARKS

Status of the Claims

Claims 1-24 are pending in the present application. Claims 1 and 12 are amended. Support for the amendment to claims 1 and 12 is found in the specification, for example, on page 12, lines 18-26, Figure 1, and Figure 2. No new matter is introduced with the amendments to claims 1 and 12. Claims 23 and 24 are added. Support for claims 23 and 24 is found in the specification, for example, on page 12, lines 18-26, Figure 1, and Figure 2. No new matter is introduced with addition of claims 23 and 24.

Claims 1-4, 6, 10-15, 17 and 21-22 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by White *et al.* Claims 1-6 and 12-17 stand rejected under 35 U.S.C. § 102(b) for allegedly being anticipated by Korte *et al.* Claims 1-6 and 12-17 stand rejected under 35 U.S.C. § 102(b) for allegedly being anticipated by Entezami *et al.* Claims 7-8 and 18-19 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over White *et al.*, Korte *et al.* and Entezami *et al.* in view of Schmitz *et al.* The rejections will be addressed in the order they were raised in the Office Action.

The Invention

As presently claimed, the present invention provides methods of separating individual phospholipids from a phospholipid mixture using a single TLC migration on a single TLC plate. The methods of separation result in highly resolved, discrete, detectable spots that can be individually detected and quantitative.

1. Rejection under 35 U.S.C. § 102(b)

Claims 1-4, 6, 10-15, 17 and 21-22 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by White *et al.* Claims 1-6 and 12-17 are rejected under 35

U.S.C. § 102(b) as allegedly anticipated by Korte *et al.* and Entezami *et al.* Applicants respectfully traverse the rejections.

As the Examiner is aware, "a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." See MPEP § 2131 (quoting *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987)). Applicants respectfully submit that none of the cited references disclose each claim element as set forth in claims 1 and 12, the two independent claims.

1.1 White et al. fails to meet Applicants' claim element of "allowing said phospholipid mixture to migrate up said TLC plate until said individual phospholipids are resolved into discrete, detectable spots".

The Examiner asserts that White *et al.* anticipates Applicants' claimed invention by teaching a method for separating target molecules using one-dimensional TLC. The Examiner states that the separated phospholipids can be detected by staining followed by exposure to ultraviolet light.

Applicants respectfully assert that White *et al.* does not expressly or inherently describe all of the elements set forth in claims 1-4, 6, 10-15, 17 and 21-22. Claims 1 and 12 now recite "allowing said phospholipid mixture ***to migrate up*** said TLC plate ***until said individual phospholipids are resolved into discrete, detectable spots*** such that all said individual phospholipids present in said phospholipid mixture can be individually detected." To anticipate Applicants' claimed invention, a disclosed method must allow a phospholipid mixture to migrate up the TLC plate ***until*** the phospholipids are separated. A method, such as that of White *et al.*, that stops migration of a phospholipid mixture before separation is achieved fails to meet this claim element.

In contrast to Applicants' claimed method, White *et al.* teaches a method of separating a phospholipid mixture using a "multiple one dimensional TLC (MOD-TLC)" (see page 9, lines 19-26). According to White, *et al.*, MOD-TLC is a process in which a mixture is allowed to migrate up the TLC plate with a first mobile phase, the

migration is stopped by fully drying the plates with a hair drier, then the mixture is allowed to migrate up the TLC plate a second time with a second mobile phase (see p.19, lines 14-26).

On page 9, lines 14-17, White *et al.* teaches that the first migration alone is insufficient to fully resolve a phospholipid mixture. White *et al.* states that MOD-TLC is advantageous over prior single migration methods "in its ability to effectively separate target molecules, especially biological lipids. This advantage is due, at least in part, to the ***combination of multiple resolutions*** in a single dimension and the specific solvents utilized" (see page 9, lines 15-17). Therefore, White *et al.* teaches that effective separation does not occur unless a combination of multiple resolutions is used wherein the first migration is halted and subsequent migrations are performed. Thus, the method of White *et al.* halts migration of a phospholipid mixture before the "phospholipids present in said phospholipid mixture can be individually detected." Therefore, the teachings of White *et al.* fails to anticipate Applicants' claimed invention which requires allowing a phospholipid mixture to migrate up the TLC plate "***until*** said individual phospholipids are resolved into discrete, detectable spots."

In summary, White *et al.* teaches the use of a multiple migration system. This multiple migration TLC method fails to meet Applicants' claim limitation of "allowing said phospholipid mixture to migrate up said TLC plate ***until*** all said individual phospholipids are resolved into discrete, detectable spots."

1.2 Korte et al. fails to meet Applicants' claim element of "such that all said individual phospholipids present in said phospholipid mixture can be individually detected".

The Examiner asserts that Korte *et al.* anticipates Applicants' claimed invention by teaching a method for separating phospholipids using one-dimensional TLC in one direction. The Examiner states that the separated phospholipids can be detected and visualized.

The Applicants respectfully assert that Korte *et al.* does not expressly or inherently describe all of the elements set forth in claims 1-6 and 12-17. Claims 1 and 12 recite methods wherein "said individual phospholipids are resolved into discrete, detectable spots such that ***all said individual phospholipids*** present in said phospholipid mixture ***can be individually detected***." To anticipate Applicants' claimed invention, ***all*** the individual phospholipids must be sufficiently separated to allow ***individual detection***. A disclosed method that fails to separate all the individual phospholipids such that they can be individually detected does not meet this claim element.

In the specification, Applicants teach that accurate individual detection of a phospholipid requires tight, non-overlapping analyte spots. The specification recites:

> The most important aspect of quantitation is the degree of separation and tightness of the analyte spots following resolution. Analyte spots that are streaked or smeared and those that are overlapping into adjacent analytes cannot be accurately quantified.

(see page 12, lines 18-20). After reading the above passage, one skilled in the art would recognize that separation methods resulting in overlapping analyte spots cannot be individually detected. Thus, the skilled artisan would reasonably understand that the claim term "individually detected" requires non-overlapping analyte spots.

The present invention provides exemplary separations of a phospholipid mixture (see, e.g., **FIG. 1** and **FIG. 2**). The exemplary separations result in non-overlapping analyte spots that allow the separated phospholipids to be individually detected. For example, **FIG. 2** shows non-overlapping analyte spots as measured in a PhosphorImager® scan of a TLC plate. The peaks are clearly separated and non-overlapping. The phospholipid mixture separated in **FIG. 2** contains phosphatidic acid (20), phosphatidylethanolamine (22), phosphatidylserine (24), phosphatidylinositol (26), phosphatidylcholine (28), and sphingomyelin (30).

In contrast, the method of Korte *et al.* results in overlapping analyte spots. For example, Fig. 3 in the Korte *et al.* reference presents a radiometric scan of a TLC plate. Several peaks are clearly overlapping and not individually detectable. For example, the peak representing phosphatidylserine (PS) is shown as a shoulder of the

peak representing phosphatidylinositol (PI). In contrast, Applicants' **FIG. 2** shows the same two phospholipids, PS and PI, as fully resolved, non-overlapping peaks.

In summary, the method of Korte *et al.* fails to separate PS and PI such that PS and PI can be individually detected. Therefore, the method of Korte *et al.* does not anticipate the Applicants' claimed invention because it does not meet the claim element of "individually detected."

1.3 Entezami et al. fails to meet Applicants' claim element of "such that all said individual phospholipids present in said phospholipid mixture can be individually detected".

The Examiner asserts that Entezami *et al.* anticipates Applicants' claimed invention by teaching a method for separating phospholipids using TLC. The Examiner states that the separated phospholipids can be scanned and detected following separation.

Applicants respectfully assert that Entezami *et al.* does not expressly or inherently describe all of the elements set forth in claims 1-6 and 12-17. As discussed above with the Korte *et al.* reference, claims 1 and 12 recite methods wherein "said individual phospholipids are resolved into discrete, detectable spots such that ***all said individual phospholipids*** present in said phospholipid mixture ***can be individually detected***." A method, such as that taught by Entezami *et al.*, that fails to separate all the individual phospholipids such that they can be individually detected does not meet this claim element.

As discussed with the Korte *et al.* reference, Applicants have taught in the specification that accurate individual detection of a phospholipid requires tight, non-overlapping analyte spots (see page 12, lines 18-20). In addition, the specification contains exemplary separations resulting in non-overlapping analyte spots that allow the separated phospholipids to be individually detected (see, e.g., **FIG. 1** and **FIG. 2**).

By contrast, the method of Entezami *et al.* results in overlapping analyte spots. For example, in Fig. 1, Entezami *et al.* presents a densitometric chromatogram of a TLC plate. Several peaks are clearly overlapping and not individually detectable. For

example, the figure shows a peak representing phosphatidylserine (PS) that overlaps with the peak representing phosphatidylcholine (PC). In contrast, Applicants' **FIG. 2** shows the same two phospholipids, PS and PC, as fully resolved. Therefore, because the method of Entezami *et al.* fails to separate, for example, PS and PI such that PS and PI can be individually detected, the method does not anticipate the Applicants' claimed invention.

In conclusion, the methods of Entezami *et al.* do not meet Applicants' claim element requiring that all the individual phospholipids are separated such that they can be individually detected.

2. Rejections under 35 U.S.C. § 103(a)

The rejection of claims 7-8 and 18-19 for allegedly being obvious over White *et al.*, Korte *et al.* and Entezami *et al.* in view of Schmitz *et al.* is respectfully traversed.

2.1 Schmitz et al. teaches away from Applicants' claimed invention

A prior art references must be considered in its entirety, including portions that teach away from the claimed invention (see MPEP § 2141.02). In addition, it is improper to combine references where the references teach away from their combination (see MPEP § 2145, *In re Grasseli*, 218 USPQ 769, 779 (Fed. Cir. 1983)). Applicants respectfully submit that Schmitz *et al.* teaches away from the Applicants' claimed invention.

As explained above, Applicants' claimed invention allows a mixture of phospholipids to migrate up a TLC plate until they are ***individually detectable***. Therefore, Applicants' claimed methods separate ***all*** the individual phospholipids of a mixture using a ***single*** TLC migration on a single TLC plate. In contrast, Schmitz *et al.* teaches that a method using a single TLC plate is inadequate to separate all the individual phospholipids in a mixture. More specifically, Schmitz *et al.* teaches that a single TLC plate is inadequate to separate neutral phospholipids from a phospholipid mixture. Schmitz *et al.* states:

> In our experience, one-dimensional separation of neutral lipids and phospholipids on the same plate can cause contamination of the separate spots by other lipids. In addition, there are calibration problems due to large differences in concentration between individual lipid classes . . . . We conclude, therefore, that the quantitatative analysis of neutral and phospholipids in natural mixtures using one-dimensional HPTLC should be done on ***separate plates*** to reduce overlapping of lipid compounds.

(see page 77, lines 20-29, emphasis added).

In view of the language quoted above, Schmitz *et al.* teaches away from Applicants' invention. Claims 1 and 12 recite methods of using a single TLC plate, not multiple TLC plates, to separate all the individual phospholipids of a mixture, including neutral phospholipids. Schmitz *et al.* states that such a technique results in contamination of the spots by other phospholipids and expressly recommends the use of multiple TLC plates to remedy the deficiencies of techniques relying on a single plate. Applicants' **FIG. 2** demonstrates that the neutral phospholipid phosphatidylcholine (PC) is individually separated from a mixture comprising charged phospholipids using a single TLC plate. This directly contradicts the teachings of Schmitz *et al.*, which recommends the use of separate TLC plates to resolve neutral phospholipids, such as PC, from a charged phospholipids.

In summary, Schmitz *et al.* teaches away from Applicants' claim element of separating ***all*** the individual phospholipids of a mixture using a ***single*** TLC plate. Therefore, it is improper to combine White *et al.*, Korte *et al.* and Entezami *et al.* with Schmitz *et al.* to make an obviousness rejection. MPEP § 2145.

2.2 A prima facie case of obviousness has not been established

In order to establish a prima facie case of obviousness, the rejection must demonstrate that (1) there is a suggestion or motivation in the prior art to modify or combine the reference teachings; (2) there is a reasonable expectation of success; and (3) the cited references teach all the claimed elements. MPEP § 2143; *In re Vaeck*, 20

USPQ2d 1438 (Fed. Cir. 1991). Applicants respectfully submit that the three criteria have not been met.

2.2.1 There is no suggestion or motivation in the prior art to modify or combine the reference teachings

The Schmitz *et al.* procedure using multiple TLC plates purports to provide "rapid separation and quantification of the major tissue and lipoprotein lipids with a high degree of sensitivity, precision, and accuracy." Schmitz *et al.* does not disclose or suggest that there is any deficiency in the disclosed method that could be remedied by the use of a single TLC plate system. In fact, as set forth above, Schmitz expressly teaches against the use of a method relying upon a single TLC plate.

The White *et al.* procedure purports to provide "sensitive and quantitative separation and detection" of phospholipids using a multiple migration system (see page 4, lines 20-21). White *et al.* does not disclose or suggest that there is any deficiency in the disclosed multiple migration system that could be remedied by the use of a single migration system such as that claimed by Applicants.

The Korte *et al.* reference states that "this method is well suited for the separation of relatively large amounts of endogenous or radiolabeled lipids and fatty acids form extracts of biological fluids . . ." (see page 53, lines20-22). Korte *et al.* does not disclose or suggest that the resolution of the analyte spots requires improvement or that it could be improved using the Applicants' claimed TLC method.

Similarly, Entezami *et al.* purports to "adequately separate the major phospholipids" (see page 330, lines 34-35). Again, there is no disclosure or suggestion in Entezami *et al.* that the resolution of the analyte spots should be improved or could be improves using Applicants' invention.

As the Examiner is aware, the motivation to modify or combine references ***must be based on what is desirable, not just on what is feasible***. *Winner International Royalty Corp. v. Wang*, 98-1553, page 17 (Fed. Cir., Jan. 27, 2000). Moreover, to avoid the pitfall of hindsight, the Examiner must "***identify specifically***...the reasons one of ordinary skill in the art would have been motivated to select the references and combine

them." *In re Rouffet* 47 USPQ2d 1453, 1459 (Fed. Cir. 1998). The Examiner has not provided a reason as to why one of skill would be motivated to combine White *et al.*, Korte *et al.*, Entezami *et al.*, and Schmitz *et al.*

2.2.2 There is no reasonable expectation of success

Applicants respectfully submit that, even if there were some motivation to combine the cited references, and the references disclosed each element of the present claims, the combination would not provide the necessary reasonable expectation of success. Regarding the method set forth in both claims 1 and 12, one of skill would expect that allowing a phospholipid mixture to migrate up a single TLC plate would ***not*** result in individually detectable phospholipids.

As explained above, Schmitz *et al.* teaches away from Applicants' claim element of separating ***all*** the individual phospholipids of a mixture using a ***single*** TLC plate. Schmitz *et al.* teaches that a single TLC plate is inadequate to separate neutral phospholipids from a phospholipid mixture. Rather than using a single TLC plate, Schmitz *et al.* teaches the use of separate TLC plates to resolve a mixture of phospholipids. Therefore, after reading Schmitz *et al.*, one skilled in the art would not expect to be successful in separating a phospholipid mixture using Applicants' claimed invention.

Similarly, White *et al.* teaches that a first migration alone is insufficient to fully resolve a phospholipid mixture. Because White *et al.* teaches that effective separation does not occur unless a multiple resolutions are used, one skilled in the art would not expect to successfully separate a phospholipid mixture using Applicants' single migration methods.

In addition, as discussed above, the methods of Korte *et al.* and Entezami *et al.* do not separate all the phospholipids in a mixture such that they are individually detectable. There is no suggestion in Korte *et al.* or Entezami *et al.* that a allowing a mixture of phospholipids to migrate up a TLC plate would result in individually detectable phospholipids. Therefore, after examining both Korte *et al.* and Entezami

et al., one of skill would not expect all the phospholipids in a mixture to be individually detectable using a TLC separation method.

In summary, after examining White *et al.*, Korte *et al.*, Entezami *et al.* and Schmitz *et al.*, one skilled in the art would not be motivated to use a single TLC plate with a single migration to separate a mixture of phospholipids such that all the phospholipids are individually detectable.

2.2.3 The Art of Record Fails to Teach Each Claim Element

Applicants' claimed method recites allowing a phospholipid mixture to migrate up a TLC plate until individual phospholipids are separated from the phospholipid mixture such that all the individual phospholipids present in the phospholipid mixture can be individually detected. None of the references now of record discloses or suggests a method wherein a mixture of phospholipids are allowed to migrate up a TLC plate until they are individually detectable. As each of the elements of the claims has not been identified in the art of record, a proper *prima facie* case of obviousness cannot be set forth relying on the art of record.

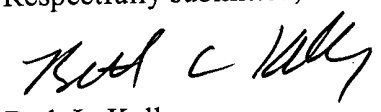
As discussed above, the White *et al.*, Korte *et al.*, Entezami *et al.*, and Schmitz *et al.* fail to teach all the elements of Applicants' claims 1 and 12. White *et al.* teaches the use of a multiple migration system which fails to meet Applicants' claim limitation of "allowing said phospholipid mixture to migrate up said TLC plate ***until*** said individual phospholipids are separated." Korte *et al.* and Entezami *et al.* fail to teach Applicants' claim element requiring that all the individual phospholipids are separated such that they can be ***individually detected***. Finally, Schmitz *et al.* fails to teach the use of a single TLC plate to separate all the phospholipids from a phospholipid mixture.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Once Amended) A method for the simultaneous quantitative and qualitative determination of individual phospholipids in a phospholipid mixture comprising:

dissolving said phospholipid mixture in at least one extraction solvent;

applying said at least one extraction solvent having said phospholipid mixture dissolved therein to a thin layer chromatography (TLC) plate;

placing said TLC plate having said phospholipid mixture applied thereto into an elution solvent mixture; and

allowing said phospholipid mixture to migrate up said TLC plate in one direction until said individual phospholipids are resolved into discrete, detectable spots [separated from said phospholipid mixture] such that all said individual phospholipids present in said phospholipid mixture can be individually detected.

12. (Once Amended) A method for the quantitative determination of individual phospholipids in a phospholipid mixture comprising:

dissolving said phospholipid mixture in at least one extraction solvent;

applying said at least one extraction solvent having said phospholipid mixture dissolved therein to a thin layer chromatography (TLC) plate;

placing said TLC plate having said phospholipid mixture applied thereto into an elution solvent mixture; and

allowing said phospholipid mixture to migrate up said TLC plate in one direction until said individual phospholipids are resolved into discrete, detectable spots [separated from said phospholipid mixture] such that all said individual phospholipids present in said phospholipid mixture can be individually detected.

23. (New) The method of claim 1, wherein said discrete, detectable spots are separately quantifiable.

24. (New) The method of claim 12, wherein said discrete, detectable spots are separately quantifiable.